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STRUCTURAL CHANGES IN THE SMALL INTESTINE ASSOCIATED WITH THE UPTAKE OF POLYVINYL PYRROLIDONE BY THE YOUNG FERRET, RABBIT, GUINEA-PIG, CAT AND CHICKEN

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SUMMARY

1. The entry of [^{125}I]polyvinyl pyrrolidone (PVP) of mean mol. wt. 160,000 (K. 60) into the epithelial cells of the small intestine has been measured in new-born animals of five species.

2. The distribution along the intestine of cells capable of taking up [^{125}I]PVP and the decrease and eventual cessation of uptake (closure) with increasing age have been investigated, and have been related to changes in the histological appearance of the small intestine.

3. The small intestine of the ferret took up PVP readily until 33–34 days after birth. From 34 to 37 days of age PVP uptake declined sharply and disappeared completely by 40–45 days.

4. In the ferret, unlike other species studied, some PVP was taken up by the duodenum. This continued for the first 4 weeks after birth. Thereafter PVP uptake gradually became confined to the terminal ileum.

5. In the guinea-pig, PVP uptake was limited to the first 48 hr after birth. During this period the site of uptake was progressively restricted to the terminal ileum.

6. In the rabbit, PVP could be taken up in the distal two-thirds of the small intestine for at least 20 days after birth. A decline in uptake occurred between 20 and 22 days after birth in most animals.

7. Wide individual variations were seen in the kitten, but PVP uptake was seen in some animals up to 14 days after birth.

8. Newly hatched chicks and chicks tested 48 hr after hatching did not take up PVP.

9. Histological examination of the small intestine with the light micro-

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scope demonstrated that in all species PVP uptake was associated with the presence of vacuoles in the epithelial cells of the villus.

10. In the young guinea-pig, large PAS-positive granules were seen in the macrophages of the lamina propria. These appeared to migrate through the epithelium into the intestinal lumen. The significance of this finding and its relation to macromolecular uptake remain unclear.

INTRODUCTION

The small intestine of the new-born of many mammalian species exhibits a unique permeability to macromolecular substances. The permeability is transient, but it enables maternal antibodies secreted in colostrum and milk to pass unchanged into the circulation of the suckling animal, thereby conferring a degree of passive immunity.

Quantitative measurements of antibody transfer are difficult, but estimations of the uptake of a macromolecular solute by the epithelial cells can provide considerable information about the behaviour of the neonatal intestine at this time.

The use of [^{125}I]polyvinyl pyrrolidone (PVP) of mean mol. wt. 160,000 (K. 60) has previously been found to have certain advantages for the investigation of macromolecular uptake by the neonatal rat intestine (Clarke & Hardy, 1969*a*, *b*). The uptake of the polymer by the epithelial cells can be assessed quantitatively, since it is not affected by digestive enzymes and does not pass through the cells into the circulation. In the rat, the age at which PVP uptake declines corresponds well with that at which antibody absorption ceases, and it is also possible to determine the distribution within the intestine of those cells which take up PVP.

For these reasons it was decided to investigate the ability of the young of other species to take up [^{125}I]PVP K. 60 into the intestinal wall. The species chosen were rabbit, ferret, guinea-pig, cat and chick; the results obtained will be considered in relation to previous investigations of the absorption of specific antibodies.

METHODS

Animals were obtained from laboratory stock and were removed from the mother at various ages after birth. They were fed the experimental solution by stomach tube, after which they were returned to the mother for 4 hr. The age quoted in the results refers to the age of the animals when killed at the end of the 4 hr uptake period.

The experimental solution comprised [^{125}I]PVP K. 60 in water containing 2 g unlabelled PVP/100 ml. The volumes fed to each species were: ferrets, 0·25–0·5 ml.; rabbits, 0·5–1·0 ml.; guinea-pigs, 0·5 ml.; cats, 0·5 ml.; chicks, 0·25 ml.

Measurement of uptake

Animals were killed by intraperitoneal injection of 12 % sodium pentobarbitone and a measured volume of blood was taken from the heart. The lungs were removed and placed in counting tubes. Animals in which ^{125}I was detected in blood and lungs were discarded, since this indicated misplacement of the stomach-tube during feeding (Clarke & Hardy, 1969*a*).

The [^{125}I]PVP remaining in the stomach was measured. This could be done directly in smaller individuals, but in older rabbits and ferrets the stomach and contents were homogenized in a commercial blender (Ato-mix: M.S.E.) and the [^{125}I]PVP content calculated from three representative aliquots. The entire small intestine was flushed through with 0·9 % NaCl and cut into fractions whose radioactivity was estimated separately.

The total amount of radioactivity in the wall of the small intestine was expressed as a percentage of the amount fed less the amount remaining in the stomach, i.e. a percentage of the amount deemed to have passed the pylorus and to have been available for uptake by the small intestine. This quantity will be referred to as the 'percent PVP uptake'.

Site of PVP uptake in the small intestine

To facilitate comparison between species this has been represented as described for rats by Clarke & Hardy (1969*a*). Thus in Text-figs. 2, 4 and 6 the width of each bar represents the length of the gut fraction relative to that of the whole intestine. The area of each bar represents the net radioactivity in that gut fraction as a percentage of the total radioactivity which had passed the pylorus:

$$\text{Relative PVP uptake} = \frac{\text{net counts/min of fraction}}{\text{total net counts/min passed pylorus}} \times \frac{\text{length of small intestine}}{\text{length of fraction}}.$$

Estimation of radioactivity

Samples were analysed in a Nuclear-Chicago type 4222 Auto-gamma system to an accuracy of better than 1 %.

Histology

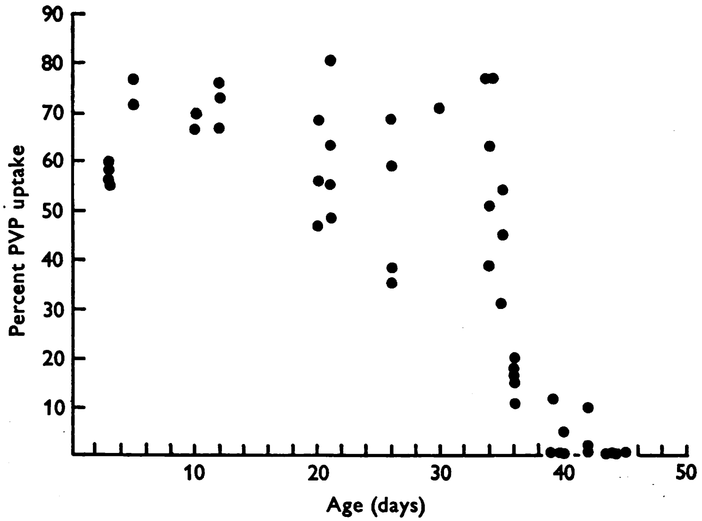
The radioactivity of some intestinal fractions was measured with the specimens in tubes containing 4 ml. Bouin's fluid. Control experiments showed that the measurement of radioactivity was unaffected by the presence or absence of Bouin's fluid in the tube. Paraffin sections were cut at 10 μm, and stained with suitable combinations of the following: Heidenhain's Iron Haematoxylin, Mayer's Haemalum, Van Gieson, Alcian Blue and the Periodic Acid–Schiff (PAS) reaction.

RESULTS

For each species studied four aspects of the results obtained will be considered: first, the effect of age on PVP uptake; secondly, the longitudinal distribution (within the small intestine) of PVP uptake; thirdly, the histological structure of the small intestine during the period of PVP uptake; and fourthly, the correlation of the histological appearances with the ability of the intestine to take up PVP.

Ferret

Individual ferrets (forty-eight) from a total of twenty-five litters were fed [^{125}I]PVP at ages ranging from 3 to 45 days after birth; the results are summarized in Text-fig. 1. During the first 33–34 days after birth the small intestine usually took up between 40 and 80% of the [125I]PVP which passed the pylorus. Between 34 and 37 days there was a dramatic reduction in PVP uptake, so that less than 15% entered the wall of the small intestine in animals older than 36 days, and by 40–45 days after birth uptake had fallen to the negligible levels seen in the adult.

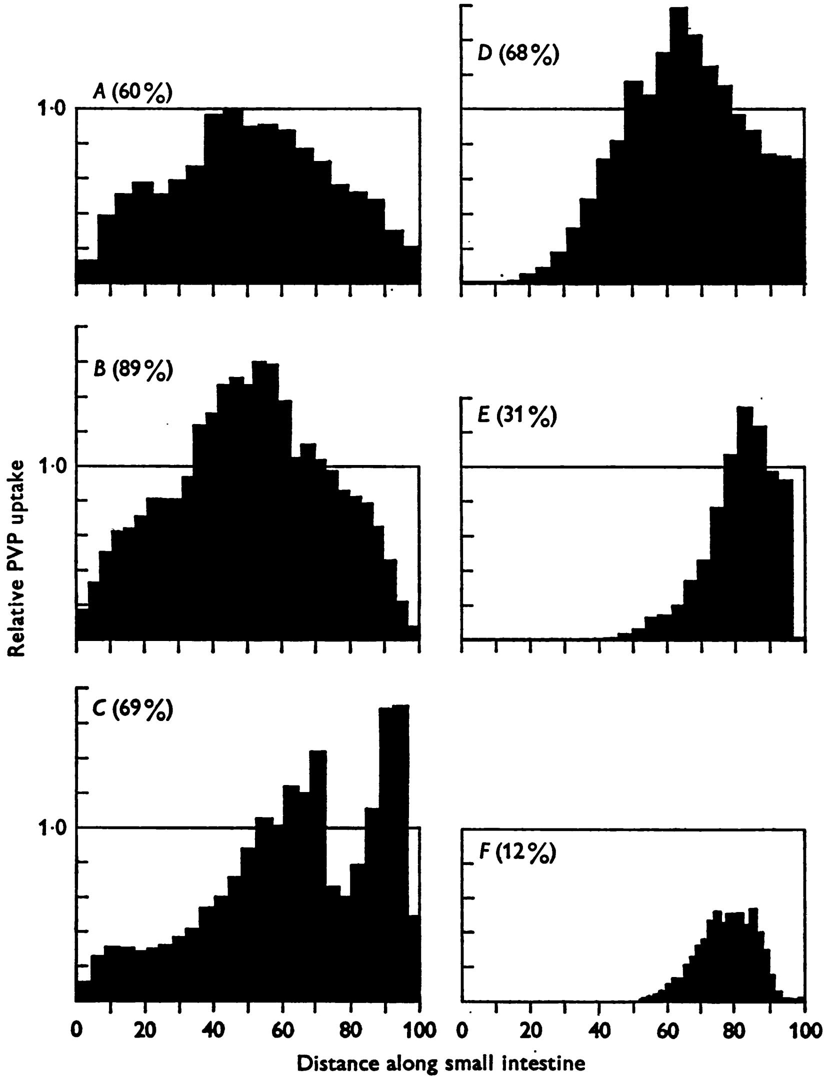


Text-fig. 1. The changes with increasing age in the uptake of [125I]PVP by the small intestine of the ferret. Ordinate: percent PVP uptake. Abscissa: age in days.

The longitudinal distribution of [125I]PVP uptake in the small intestine of ferrets of different ages is shown in Text-fig. 2. Two features are apparent: first, there was a progressive reduction in uptake by cells of the proximal half of the intestine without gross change in total PVP uptake—a process essentially complete within the first 30 days. Secondly, after 30 days there was a decrease in PVP uptake in the distal small intestine, which was associated with the decline in total PVP uptake.

A total of sixty specimens from twenty-seven individuals were examined histologically. On the day of birth, the villus epithelium of the small intestine of the suckled new-born ferret was largely composed of vacuolated cells which contained large ($< 10\,\mu m$) globules of PAS-positive material. The vacuoles and globules were absent from cells at the bottom of the villus, but increased in size towards the top of the villus (Pl. 1, fig. 1). The vacuoles were usually apical to the nucleus, but were occasionally seen below it in these young animals.

On the second day of life, vacuolated cells occupied most of the villus throughout almost the whole length of the small intestine, apart from the proximal duodenum, where the only vacuolated cells were a few empty ones at the top of the villus. By the third day, small globules were seen in the



Text-fig. 2. Changes with age in the longitudinal distribution of [^{125}I]PVP uptake in the small intestine of the ferret 4 hr after feeding. *A*, 3 days; *B*, 9 days; *C*, 26 days; *D*, 30 days; *E*, 35 days; *F*, 39 days. Ordinate: relative PVP uptake. Abscissa: distance along small intestine; 0, pylorus; 100, ileocaecal valve. Figures in parentheses indicate total uptake of [^{125}I]PVP as a percentage of [^{125}I]PVP which had passed the pylorus. Uniform and total uptake of all PVP passing the pylorus would be represented by the area enclosed by the rectangle.

vacuoles in the proximal intestine, while more distally the vacuoles appeared virtually empty, with small granular deposits on their walls. These cells were seen for about the next 4 weeks and some contained moderate amounts of PAS-positive material; the presence or absence of this material could not be related to recent suckling, to starvation of the young ferret, or to feeding PVP by stomach tube.

The villi of the proximal part of the small intestine remained clothed with heavily vacuolated cells up to about the thirtieth day of life, while those in the distal part remained similarly clothed up to about the thirty-fifth day. During the following 3 or 4 days, however, these cells, still heavily vacuolated, became progressively restricted to the top of the villus (Pl. 1, figs. 2 to 4); they persisted a little longer in the mid-ileum than in the terminal ileum and were rarely seen on or after the thirty-ninth day of life.

The uptake of PVP by any segment of the small intestine of the ferret was thus invariably associated with the presence of vacuolated cells in that segment.

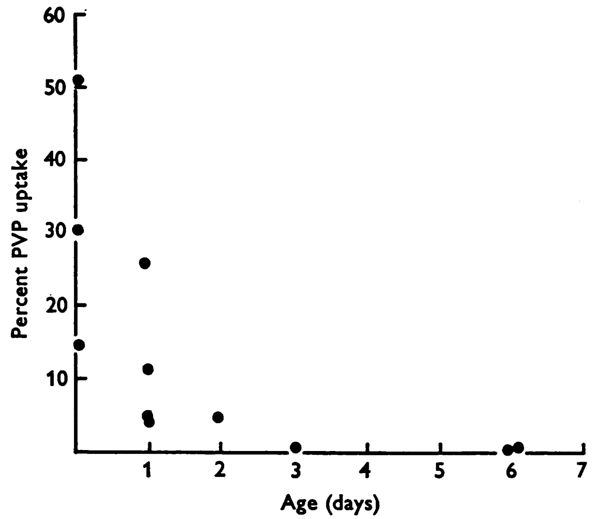
Guinea-pig

The ability of the small intestine of the young guinea-pig to take up [^{125}I]PVP was restricted to the first 48–72 hr after birth (Text-fig. 3). The uptake in the three animals fed PVP within 1 hr of birth showed a wide variation, which may have been related to maternal attention: the animal which absorbed 51 % was groomed by the mother and had suckled liberally; the other two were litter-mates, who were not dried by the mother and who were found to have no milk in the stomach post mortem.

The change in the distribution of cells taking up PVP with age is illustrated in Text-fig. 4, which shows three litter-mates fed PVP at 1, 21 and 45 hr after birth.

In the animal fed [^{125}I]PVP after being allowed to suckle for 1 hr after birth, the polymer was taken up to a variable extent by the distal 90 % of the small intestine. The decline in uptake in the terminal 25 % of the intestine could not be explained by a slow transit of PVP along the intestine, since 30 % of the polymer entering the small intestine had passed completely through it and was recovered from the large intestine at the end of the experiment. By 21 hr after birth PVP uptake was restricted to the terminal 70 % of the small intestine and the uptake by individual fractions in this area was considerably reduced. This process continued until in the 45-hr-old animal uptake was limited to the final 30 % of the intestine, each fraction of which took up only small amounts of PVP.

Histological examination was made of fifty-seven specimens from thirty individuals between birth and 7 days of age. No striking differences were noted between different zones of the same intestine and no consistent differences appeared to result from feeding PVP or Blue Dextran by stomach tube. The description that follows is of the histological appearance of the junction of the third and fourth quarters of the small intestine.

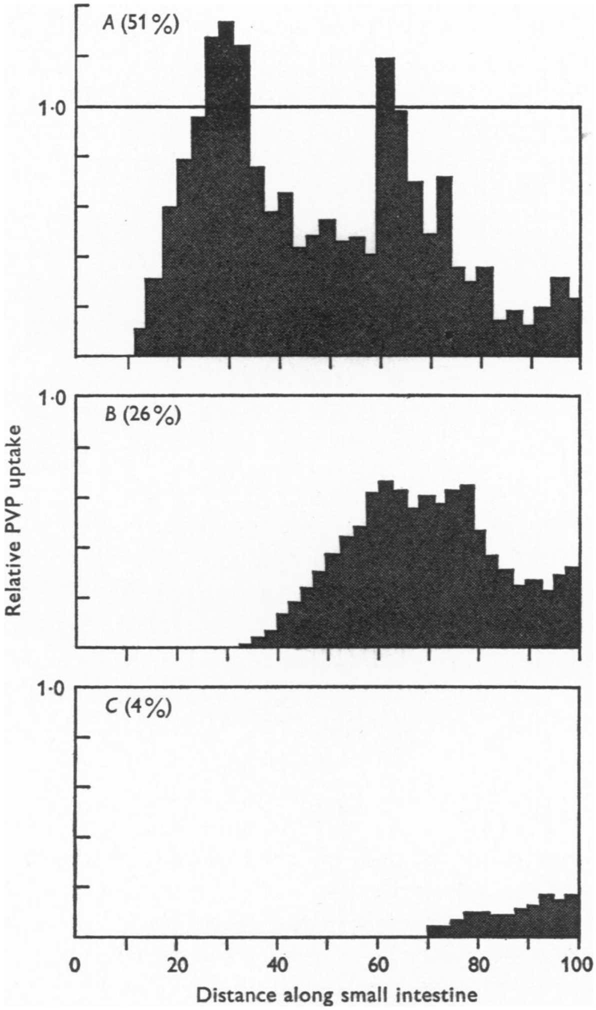


Text-fig. 3. The changes with increasing age in the uptake of [^{125}I]PVP by the small intestine of the guinea-pig. Ordinate and abscissa as Text-fig. 1.

Development could be divided into four overlapping phases. In the suckled new-born guinea-pig, the epithelium of the villus contained large ($< 20\ \mu m$) supranuclear vacuoles; these occasionally contained PAS-positive vesicles or globules of varying sizes, and PAS-positive material was sometimes seen in the lacteal (Pl. 2, fig. 1). The vacuolated appearance became less obvious, and had disappeared by about 36 hr in this region, but persisted until about 42 hr in the terminal ileum. Disappearance of the vacuoles appeared to be due both to replacement by non-vacuolated cells from below, and to replacement of the vacuole in the cell by cytoplasmic vesicles. Thus the second phase was characterized by vesicles within the epithelial cells. These were small (1–2 μm diam.), often variable in size, and were more prominent at the top of the villus than at the base. They were present to a greater or lesser degree throughout the first week of life.

The third phase comprised the accumulation of larger (5 μm diam.) PAS-positive globules in the lamina propria (Pl. 2, figs. 2 to 4). This began at 18–24 hr of age, and was at its peak at 48 hr. In specimens from two younger animals there appeared to be PAS-positive material in the lacteals, but during this second phase the globules were probably in macrophages, for the following reasons: (i) they were multifocal in the villus, while the lacteal was single and central; (ii) they were rarely found in the lower half of the villus; (iii) they were in circumscribed groups, often associated with a rounded nucleus; and (iv) lacteals which did not contain globules could be positively identified.

The fourth phase comprised the migration of globule-laden macrophages into the epithelium (Pl. 2, figs. 3, 4). This began after about 48 hr, and was still present in the oldest animal examined, at 7 days. It was not clear



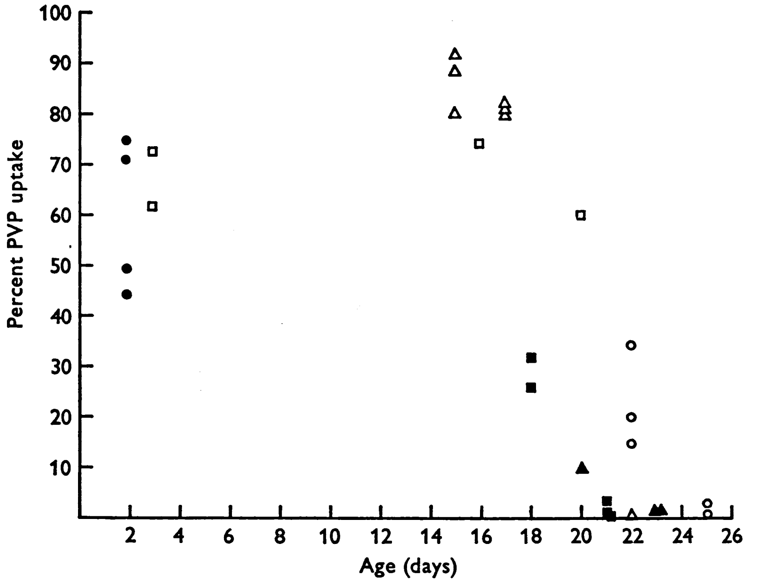
Text-fig. 4. Changes with age in the longitudinal distribution of [^{125}I]PVP uptake in the small intestine of the guinea-pig 4 hr after feeding. Age when fed; *A*, 1 hr; *B*, 21 hr; *C*, 45 hr. Annotation as in Text-fig. 2.

whether the macrophages burst through the tight junction between the epithelial cells, or whether they remained intra-epithelial until the epithelial cells were sloughed from the top of the villus.

The period of PVP uptake coincides with the period of obvious vacuolation of the epithelial cells, and both phenomena had virtually disappeared within 48 hr of birth.

Rabbit

The results of measurements of [^{125}I]PVP uptake by twenty-eight rabbits up to 25 days after birth (six litters) are shown in Text-fig. 5. It can be seen that the ability to take up PVP was retained for at least 20 days after birth. The time of closure showed some variation between litters, although results within a litter were consistent. Closure in most litters occurred at 20–22 days after birth, although individuals from one litter

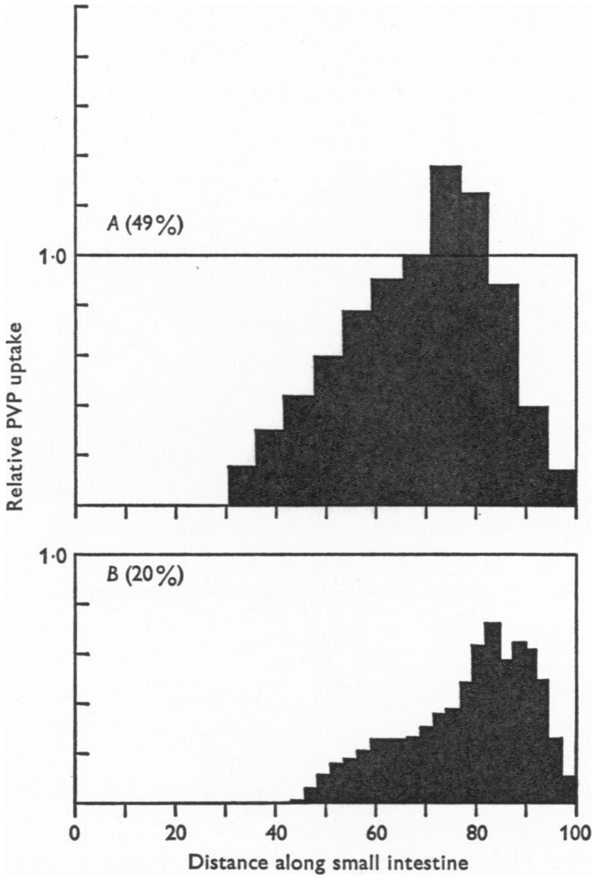


Text-fig. 5. The changes with increasing age in the uptake of [^{125}I]PVP by the small intestine of the rabbit. Individuals from the same litter share a common symbol, e.g. ●. Ordinate and abscissa as Text-fig. 1.

were still taking up PVP at 22 days and closure would probably have been delayed in this litter until 24–25 days after birth. The reason for this variation between litters is not clear, but it is possibly genetic, since all animals were maintained under comparable environmental conditions.

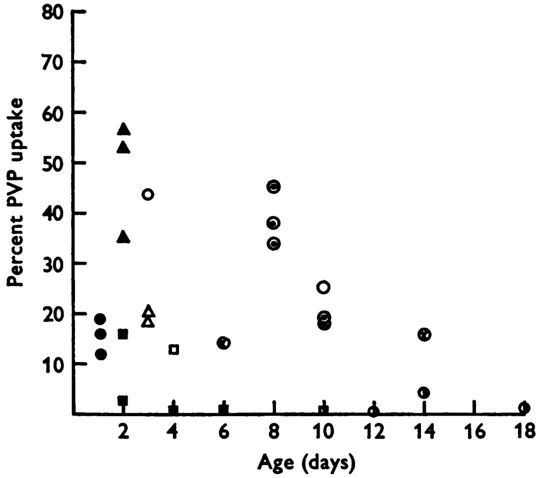
The distribution along the small intestine of cells able to take up PVP is shown in Text-fig. 6. Even in the youngest animals, PVP was not taken up in the proximal quarter of the intestine; the pattern of uptake seen in the 22-hr-old animal persisted until closure, and was similar to that previously reported in the rat (Clarke & Hardy, 1969*b*).

Histological examination was made of twenty-eight specimens from nine rabbits whose intestines had been fractionated. In the two 22-hr-old suckled rabbits, PAS-positive vesicles and globules were seen in the epithelium of the upper part of the villi in the ileum (Pl. 3, fig. 1). These were similar to those seen in suckled guinea-pigs of the same age. In older animals (15, 17, 20 and 22 days old), specimens were taken from various sites along the intestine; the histological picture was comparable with that in the rat, with large vacuoles in the apical cytoplasm of the epithelium of the ileal villi. The degree of vacuolation of the epithelium could be correlated with



Text-fig. 6. Changes with age in the longitudinal distribution of [125I]PVP uptake by the small intestine of the rabbit 4 hr after feeding. Age when fed: *A*, 22 hr; *B*, 22 days. Annotation as in Text-fig. 2.

the uptake of PVP, as in the rat, and loss of the ability of the intestine to take up PVP was associated with the progressive restriction of vacuolated cells to the top of the villus, as though the cells were being replaced from below by non-vacuolated cells incapable of taking up PVP (Pl. 3, figs. 2 to 4).



Text-fig. 7. The changes with increasing age in the uptake of [^{125}I]PVP by the small intestine of the cat. Individuals from the same litter share a common symbol, e.g. ●. Ordinate and abscissa as Text-fig. 1.

Cat

The uptake of [^{125}I]PVP K. 60 by the small intestine of twenty-six kittens tested between 1 and 18 days after birth is shown in Text-fig. 7. It will be seen that results from different litters showed wide variations, although results within a litter were more consistent. No explanation for these variations can be offered at present, although, as was suggested in the case of the guinea-pig, some of the disparity between litters may be related to differences in maternal behaviour. Those animals which were kept warm and were found to have suckled adequately absorbed more PVP than individuals of comparable age which had been less favoured by the mother. The variation in uptake between individuals was not attributable to sex differences or to their birth weight.

The pattern of PVP uptake along the intestine was similar to that seen in the rat and rabbit.

The histological picture was not as clear-cut as that seen in other species. Vacuolated cells were seen on the villi of the distal half of the small intestine, but the appearance of specimens from different animals of the same age was frequently very different. In general, the vacuoles were always apical in the cell, although not as large as those seen in rodents, for example, where the nucleus may be squashed to the base of the cell; the whole villus was rarely covered with vacuolated cells.

Much of the difficulty in histological interpretation disappeared when a correlation with PVP uptake was made, as it became clear that the apparently anomalous specimens with few or no hydropic cells were also those which took up little or no PVP. In this species, then, as in the others, vacuolation of the epithelial cells of the villus was associated with their ability to take up PVP.

Chick

No PVP was taken up by chicks fed within minutes of hatching or by chicks fed 48 hr after hatching.

No vacuolated cells were seen anywhere along the length of the small intestine of a chick killed 4 hr after hatching; nor were vacuolated cells ever seen in the small intestines of other chicks of various ages in the course of another investigation.

DISCUSSION

There is considerable variation in the ability of new-born animals from different species to absorb immunoglobulins from the colostrum and milk. In man such absorption appears to be negligible, while in other species studied the process is limited to a characteristic period which varies between the 24–48 hr typical of the ruminant (Balfour & Comline, 1962; Deutsch & Smith, 1957; McCarthy & McDougall, 1953; Pierce, 1961) and the 30–40 days during which the young hedgehog can absorb antibodies (Morris, B., 1963). Furthermore, the mechanisms of the absorptive process differ between species, so that while the young ruminant can apparently absorb a variety of large proteins, dextrans and even synthetic polymers without overt discrimination (Balfour & Comline, 1959; Pierce, 1961; Hardy, 1969*a*, *c*), the intestine of the young rodent is highly selective and will preferentially absorb proteins with specific structural configurations (Halliday & Kekwick, 1961; Morris, I. G., 1964).

The transfer to the new-born animal of maternal antibodies entails their uptake from the colostrum or milk by the villus epithelial cells, transfer across the cells and finally extrusion into the lamina propria. The termination of any one of these processes results in the cessation of antibody transfer (closure).

Previous studies in the rat (Clarke & Hardy, 1969*a*, *b*) have shown a correlation between the duration of the period after birth when [^{125}I]PVP is taken up by the villus epithelial cells and that of the reported period when antibody absorption takes place. Thus in this species it appears that it is the uptake process which limits antibody transfer. In contrast, in the pig and the goat (Clarke & Hardy, 1970*a*, *b*) [^{125}I]PVP can still be taken up by the epithelial cells after the transfer of antibody, or indeed of PVP itself, into the blood has ceased. Therefore in these two species it appears that cellular uptake of immune protein or of PVP is not the limiting factor in their transfer to the circulation.

Measurements of the duration of the period of PVP uptake in each species studied in the present investigation can be compared with previous data relating to antibody transfer, in an attempt to determine whether the cessation of antibody transfer is a consequence of failure in cellular uptake or of events within the cell.

Ferret

There have been no estimates of the duration of the period of antibody transfer in this species, although Porter (1965) reported that in the mink [^{131}I] homologous colostrum albumin and γ-globulin were transferred into the circulation of the kits for at least 8 days after birth.

Since no label was found in the blood of any ferret fed [^{125}I]PVP during the present investigation, absorption into the circulation appears to be selective in ferrets more than 3 days old. However, the histological appearance of the 1- and 2-day-old animals suggests that transfer of material from the epithelium to the subepithelial region may take place at this early age, and the possibility of PVP transfer into the blood of ferrets less than 3 days old should be investigated.

The period of 40–45 days during which PVP can be taken up by the villus epithelial cells of the ferret is the longest yet recorded for macromolecular uptake, exceeding the estimate of 30–40 days for the absorption of anti *Salmonella pullorum* agglutinins by the hedgehog reported by Morris, B. (1963). Our observations support and extend those of Williams & Beck (1969).

Rabbit

It has been found that PVP can be taken up by the intestinal epithelial cells for about 20 days after birth and that such cells have the characteristic vacuolated appearance associated in other species with the ability to take up macromolecules. These findings may appear surprising in view of the usually accepted absence in this species either of postnatal antibody transfer (Brambell, 1958) or of absorption of isotopically labelled γ-globulin (Hemmings, cited by Brambell, 1958). However, Kellner & Hedal (1953) demonstrated that γ-globulin could pass from the intestine into the circulation during the first day of suckling at least, while Kozlovskaia (1957) has shown the absorption of specific antibodies during the first 5–10 days after birth. Furthermore, Kraehenbuhl, Gloor & Blanc (1967) reported histological evidence for the presence of ferritin in the vessels of the lamina propria following the introduction of this protein into the intestinal lumen, although they were unable to demonstrate ferritin in the peripheral circulation. Graney (1968) attributes this finding of ferritin in the vessels to technical artifact. Subsequently Kraehenbuhl & Campiche (1969) have described in detail the uptake of anti-ferritin globulin and anti-peroxidase by the proximal jejunum of the new-born rabbit, although they did not determine either the longitudinal extent of cells capable of taking up these proteins or the duration of the period when such uptake was possible.

Our results are in agreement with those of Kraehenbuhl & Campiche (1969) and of Williams & Beck (1969), in that macromolecular uptake does occur in the new-born rabbit intestine and that the substances tested ([^{125}I]PVP, trypan blue, anti-peroxidase and anti-ferritin globulin) do not appear in the circulation. The reports of Kellner & Hedal (1953) and Kozlovskaia (1957), that specific antibodies pass into the circulation, may be attributed to the relative acceptability of these molecules to an intracellular selection mechanism determining extrusion from the cell, a mechanism which totally rejects such substances as PVP and peroxidase. The nature of such a selection mechanism has been the subject of speculation in the rodent (Brambell, 1966; Morris, 1968; Clarke & Hardy, 1969*a*), while Kraehenbuhl & Campiche (1969) have recently provided further evidence for lysosomal activity as a possible mechanism in the rabbit. In the latter species, the entry of macromolecular protein activates lysosomal enzymes and these authors suggest that such protein is sequestered in the lysosomes and broken down into its component amino acids. If those specific antibodies which appear to be transferred intact into the circulation escaped total digestion, such a mechanism might well explain selective absorption of proteins. However, the absence of [^{125}I]PVP in the blood following its uptake by the cell remains to be explained, since this substance is not broken down by enzymes in the gut (Hardy, 1969*b*).

The weight of evidence available at present indicates that little, if any, immune protein is transferred into the circulation of the young rabbit during suckling. In view of this, the significance of the prolonged ability of the small intestine to take up macromolecular substances such as PVP remains obscure. It would be of interest in this respect to establish whether a similar uptake of macromolecules occurs in man and other primates during early suckling, for it is generally agreed that in these species, as in the rabbit, transfer of colostrum antibodies to the circulation is insignificant or absent (Norbring, 1957; Dixon, Kuhns, Weigle & Taylor, 1959; DuPan, Scheidegger, Wenger, Koechli & Roux, 1959; Schneegans, von Muralt & Dierheimer-Vaur, 1962).

Guinea-pig

The terminal small intestine of this species can take up PVP immediately after birth, but uptake declines rapidly with age and has virtually ceased within 48–72 hr. This agrees well with the observation of Leissring & Anderson (1961) that 'complete' *Brucella abortus* agglutinins could be transferred from the intestine to the circulation during the first 3 days after birth. However, these workers also showed absorption of 'incomplete' agglutinins for at least 7 days *post partum*: an observation of considerable interest since it is the only example of significant transfer of macromolecules to the circulation after PVP uptake has ceased. However, this result may perhaps be attributable to differences between individual strains of guinea-pig because neither Jo-Keiichiro (1953) nor Barnes (1959) was able to demonstrate any post-natal transfer of specific antibodies to the suckling guinea-pig.

The striking histological feature of the small intestine of the young guinea-pig (described also by Williams & Beck, 1969) is the appearance of PAS-positive material in the lamina propria. The PAS-positive material was present whether or not macromolecular material had been fed, but appeared to enter the lamina propria via the epithelium rather than via the blood-stream because (*a*) it appeared just after the period of macromolecular uptake and (*b*) it was never seen at the base of the villus or in the blood-vessels. Unless transport from elsewhere is exceedingly rapid, one would expect to be able to see evidence of PAS-positive material in transit up the villus or in blood vessels. The PAS-positive material is sequestered into macrophages, and the macrophages subsequently migrate into the epithelium. The functional significance of this is difficult to interpret, but the appearances suggest that certain materials are taken up by, and traverse, the epithelial cells. These materials do not appear to enter the circulation but instead are phagocytosed by macrophages of the lamina propria and are subsequently excreted into the lumen of the gut.

Many of the events described occur near the limit of resolution of the light microscope, and would repay study with the electron microscope.

Cat

The variation between individuals described above was particularly noticeable in measurements of PVP uptake in the kitten. No clear explanation for the differences is available at present: the variation was not attributable to the sex or birth weight of the animals. These individual variations make difficult any systematic analysis of the results of measurements of PVP uptake, but it is clear that certain animals did take up appreciable amounts of PVP even at 10 or 14 days after birth.

Little is known about the duration of the period of antibody transfer in the suckling kitten, although colostrum antibodies apparently provide the greater part of the animal's passive immunity and are extensively absorbed during the first 24 hr after birth (Causse-Vaills, Verain & Verain, 1961; Harding, Bruner & Bryant, 1961). Miller & Ben Shaul (1965) report the absorption of bovine serum albumin and human γ-globulin by new-born kittens in amounts detectable in the serum 4 hr after feeding. Absorption of these proteins in amounts sufficient to promote an immune response, but insufficient to be measured in the plasma, was also recorded up to 25–28 days after birth: the quantities involved in this latter absorption are probably small and irrelevant to the present discussion.

In the kitten, therefore, PVP uptake can occur up to 10–14 days after birth and small traces of antigenic protein may pass across the intestine during the first 4 weeks, but the duration of the period of significant colostrum antibody transfer remains to be established.

Chick

The small intestine of the newly hatched chick does not take up PVP, nor does such uptake take place during the subsequent 48 hr. Furthermore, the intestine shows none of the histological characteristics associated with macromolecular uptake or transfer in the mammalian species studied. These results are in agreement with the observation of Brierley & Hemmings (1956) that no antibodies were absorbed from the intestine into the circulation of the chick during the first 48 hr after hatching.

The longitudinal distribution of PVP uptake in the small intestine which has been described in this paper may be compared with that reported in the rat (Clarke & Hardy, 1969*a*, *b*) and in the pig and the goat (Clarke & Hardy, 1970*a*, *b*).

The species studied fall into two groups: in one, comprising the rat, rabbit, guinea-pig, cat and goat, PVP uptake is restricted to the terminal two-thirds of the small intestine, while in the other, comprising the ferret and pig, PVP uptake, in very young animals occurs relatively uniformly throughout the entire small intestine. In the latter group, the pattern of PVP uptake comes to resemble that of the former group as the animals increase in age. The significance of the PVP uptake in the proximal small intestine seen in the pig and ferret is unknown but can be correlated with the structural changes observed in this region; these follow a different time course from that of comparable changes in the terminal ileum.

In all species studied the eventual termination of PVP uptake takes place in the ileum, and histological evidence suggests that the process may be associated with the production by the crypts of Lieberkühn of a population of more mature epithelial cells which progressively ascend the villi, replacing those vacuolated cells which can take up PVP. A detailed analysis of this transition has previously been described in the rat (Clarke & Hardy, 1969*b*).

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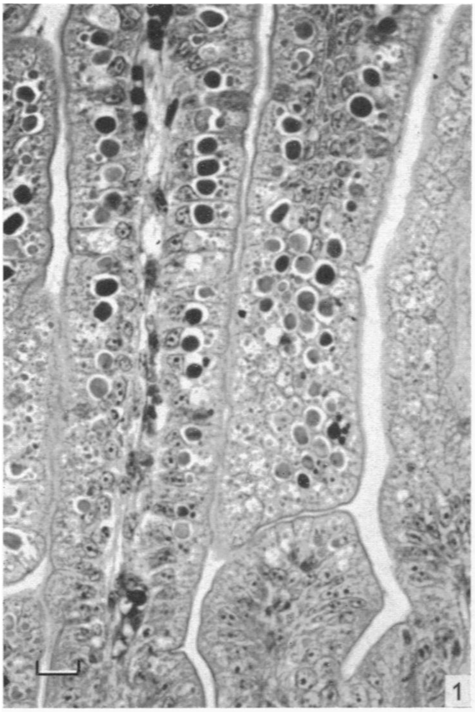
EXPLANATION OF PLATES

PLATE 1

Ferret intestine. Fig. 1: base of villi of jejunum of 2-day-old ferret; Iron Haematoxylin and Van Gieson. Scale line = 20 μm. Vacuoles contain globules which stain with Iron Haematoxylin. Figs. 2, 3, 4: mucosa of ileum of ferrets 35, 36, 39 days old respectively; Haemalum, Van Gieson and Alcian Blue. Scale line = 100 μm. Note progressive disappearance of vacuolated cells.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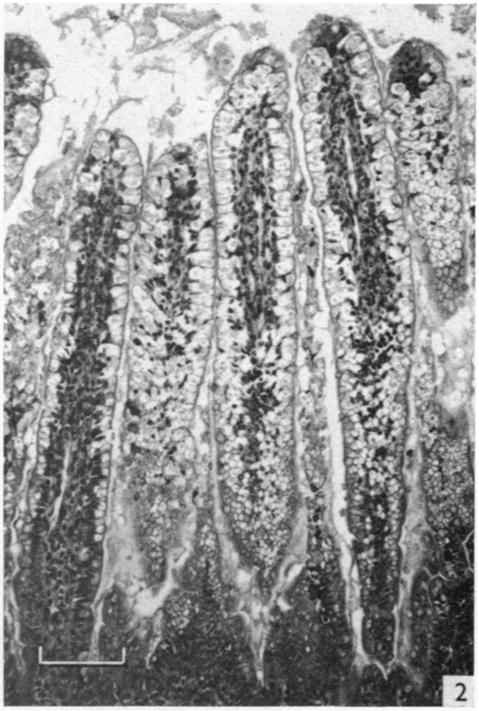
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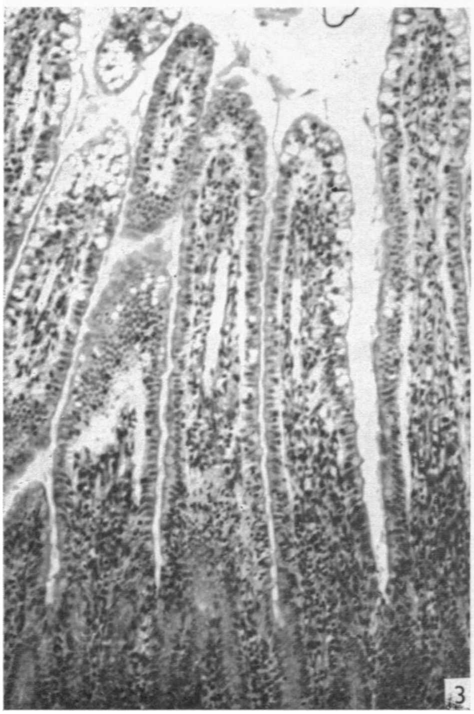
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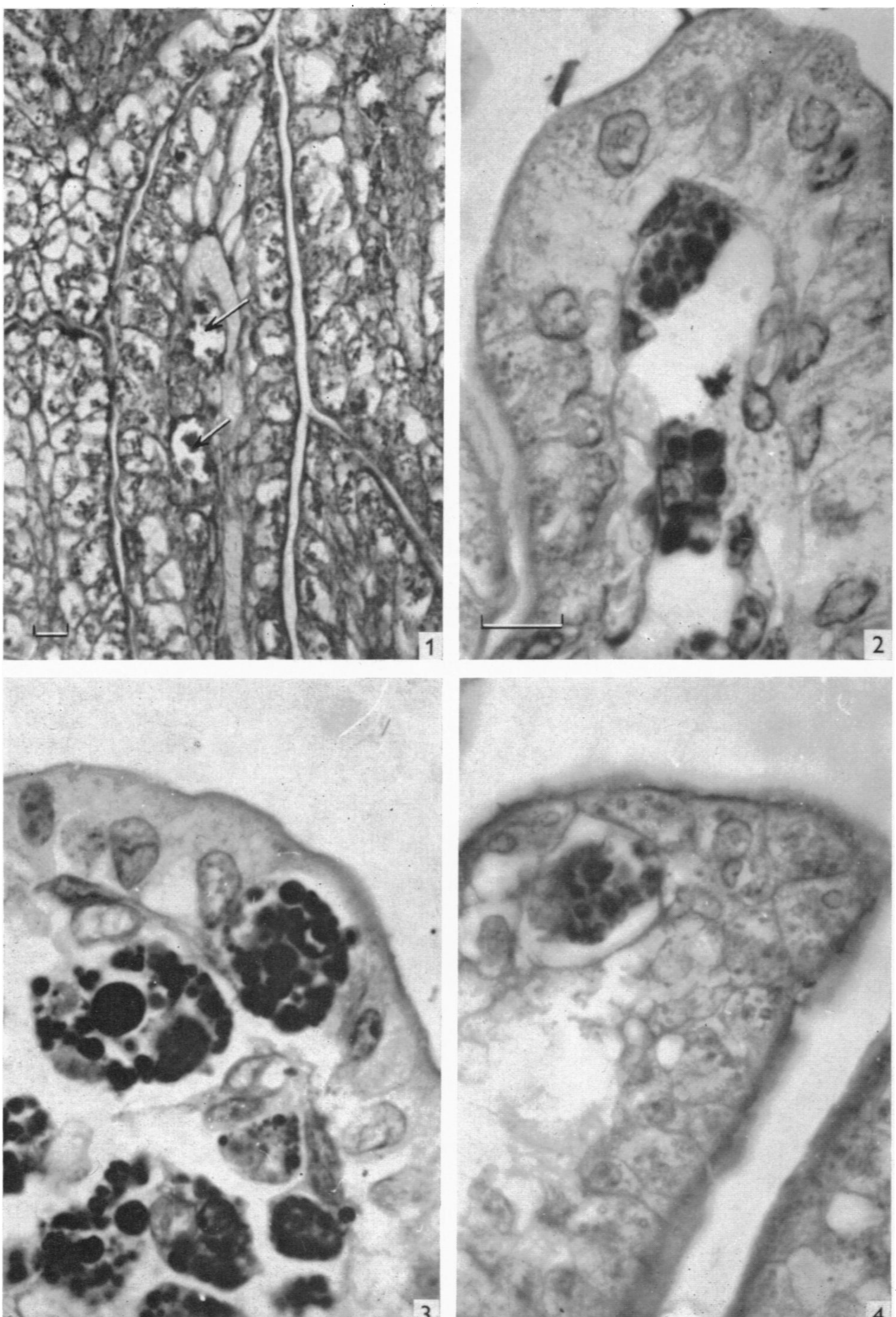

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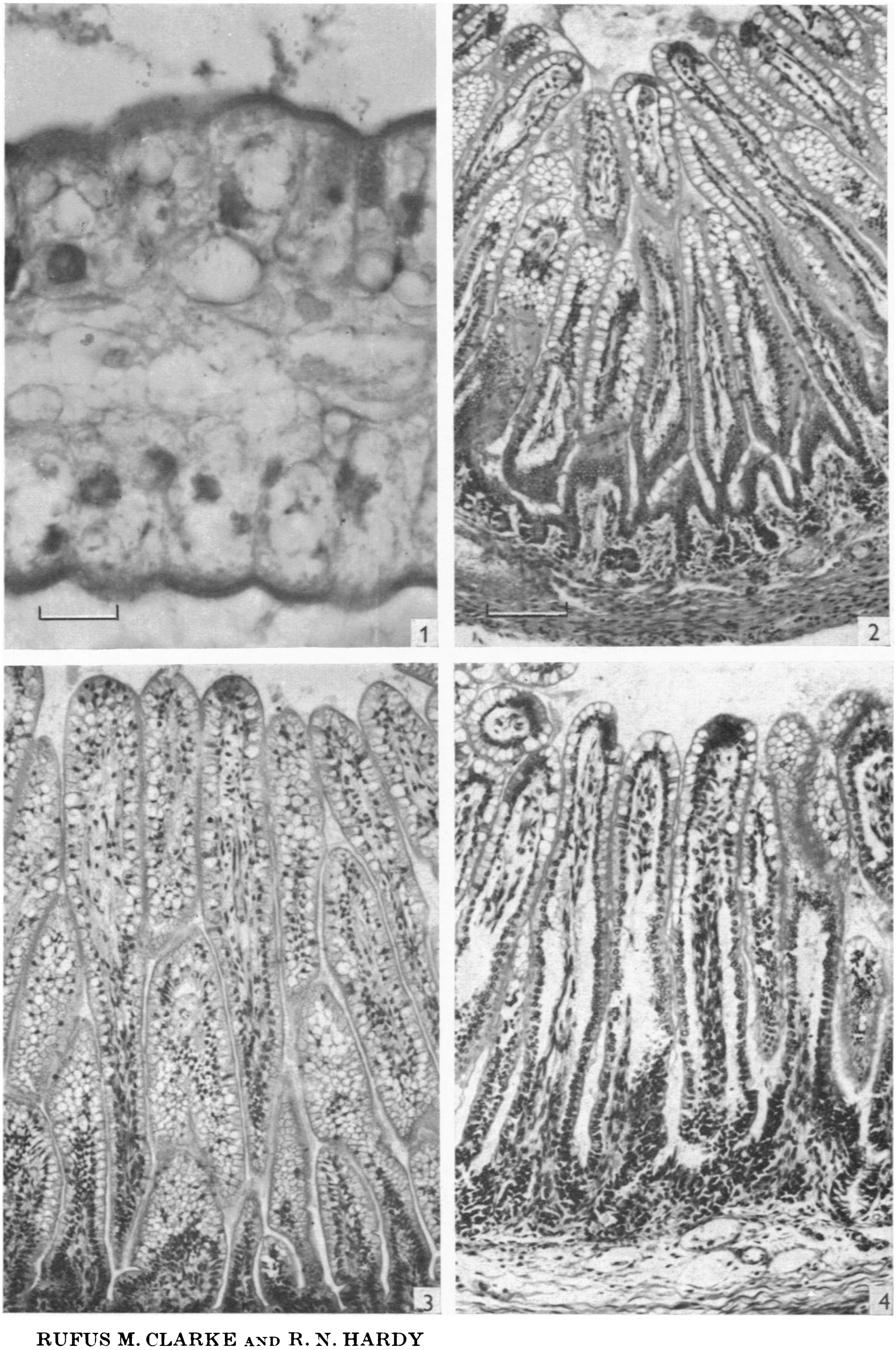

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PLATE 2

Guinea-pig intestine. All sections from junction of third and fourth quarters of guinea-pig small intestine. Fig. 1: 1 day old; PAS and Alcian Blue. Scale line = 20 μm. Extensive ragged vacuolation of epithelium, with PAS-positive material in lacteals (↙↙); Figs. 2–4: 4 days old; Haemalum and PAS. Scale line = 10 μm. Macrophages laden with PAS-positive material in lamina propria and epithelium.

PLATE 3

Rabbit intestine. Transverse sections of ileum of rabbits. Fig. 1: 1 day old; PAS and Alcian Blue. Scale line = 10 μm. Ragged epithelial vesicles with PAS-positive material. Figs. 2, 3, 4: 15, 20, 20 days old respectively; Haemalum, Van Gieson and Alcian Blue. Scale line = 100 μm. Note progressive disappearance of vacuolated cells.